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## **New approaches for imaging bacteria and neutrophils for detection of occult infections**

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# **Chapter 1**

## **Introduction**

### **Infections and molecular nuclear medicine**

Inflammatory and infectious diseases are a heterogeneous class of pathological conditions. They can be systemic or localized to one specific organ.

Inflammation and infection are not synonymous. With the term of “inflammation” we refer to a biological complex process due to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation might be considered a mechanism of innate immune system in order to remove the injurious stimuli and to initiate the healing process. If it is caused by a sterile injury, a degenerative process, or an irritant, it is a “sterile” inflammation that can be acute or chronic.

By contrast, if the cause is an exogenous pathogen such as viruses, prions, bacteria, and viroids, and larger organisms like parasites and fungi, it is a “septic” inflammation, or “infection”, that can also be acute or chronic.

Therefore, the inflammation can be usually associated to infection, but not always [1, 2].

Infectious diseases represented a serious and significant cause of morbidity and mortality worldwide until the beginning of the 20<sup>th</sup> century when chronic degenerative diseases and cancer began to dominate the scenario in developed countries. Many infections are caused by bacteria, both Gram-positive or Gram-negative, and they can already be present before leading to clinically over infection. Therefore, a rapid and accurate diagnosis is crucial for early therapeutic interventions, when the infectious process is still reversible and major damage has not yet occurred, and to discriminate the infection from an inflammatory condition. The diagnosis of bacterial infections may include several approaches: traditional methods, such as serological and microbiological procedures, despite invasive, and unconventional methods, such as molecular biology, genetic, radiological and nuclear medicine techniques. Besides, the choice of the best technique mainly depends on the type or the site of infection. Thus, the best approach is to combine traditional and unconventional methods [3].

Molecular nuclear medicine imaging offers a unique possibility to discriminate infections from inflammatory processes. Molecular imaging approaches for bacterial infections have the potential, in addition to discriminatory capabilities, to visualize the anatomical spread of infections, to identify the type of bacteria, to select the best antimicrobial therapy and to allow therapy monitoring.

Recently, the progress in understanding and knowledge of the pathophysiology of many infectious and inflammatory diseases has led to the development of several new and specific radiopharmaceuticals in order to meet clinical demand. Indeed, imaging targets in infection and inflammation may be the pathogens (bacteria, fungi), the activated endothelial cells and the involved cytokines and mediators in this process, the macromolecules that accumulate in inflamed tissues due to the increased vascular permeability and the polymorphonuclear cells (leukocytes, granulocytes) that reach the injured tissue.

The correct and best choice of which radiopharmaceutical should be used depends on the body location, tissue type of lesion (skeletal or soft tissue), patient's immune status, pharmacokinetic and properties of the radiopharmaceutical.

Currently, a plethora of imaging radiopharmaceuticals are available for non-invasive visualization of inflammations and infections, including <sup>67</sup>Ga-<sup>68</sup>Ga-citrate, [<sup>18</sup>F]FDG, radiolabelled cytokines, anti-granulocyte monoclonal antibodies, radiolabelled autologous white blood cells (WBCs) and, in particular, radiolabelled antibiotics, anti-microbial peptides and vitamins [4-6].

For example, several classes of antibiotics have been radiolabelled with <sup>99m</sup>Tc for infection imaging, particularly <sup>99m</sup>Tc-ciprofloxacin has been intensively studied, but none of developed radiopharmaceuticals has been introduced in the routine clinical practice due to controversial results between published studies [7]. During last years,

various compounds were radiolabelled with PET isotopes to discriminate septic from sterile inflammation, obtaining promising results but many candidate compounds remain confined in a pre-clinical setting needing further investigations. Despite many overall promising results, no radiopharmaceutical has been introduced into clinical practice yet. This is probably because specific and non-specific mechanisms coexist at the infection site leading to a bias in the analysis of the results. For this goal, the chapters 2 and 3 are two systematic reviews that are specifically focused on available SPECT and PET radiopharmaceuticals for infection imaging, respectively. In particular, in the chapter 2, a systematic analysis was performed to describe the main characteristics and differences among various  $^{99m}\text{Tc}$ -radiolabelled antibiotics. In the chapter 3, a systematic review of literature was performed focused on PET radiopharmaceuticals for bacterial infection imaging. In addition, each included study was evaluated by the *Quality Assessment of Diagnostic Accuracy Studies* (QUADAS) approach with the aim to assess any potential bias and variation.

Therefore, the available radiopharmaceuticals cannot be considered “infection specific” because sensitivity and specificity vary in relation to the type of infection, the type of micro-organism, the infection site and the host clinical conditions/response. When designing new studies, standardized protocols and models could ensure that time, funds, and research efforts are put to the best possible use. Indeed, the most frequent sources of bias among studies were related to animal selection and index test and showed the lack of standardization with current infection models and experimental settings. Thus, standardized protocols and consensus guidelines regarding animal models of infection are needed, preferably written by a joint technical committee [8].

For nuclear medicine imaging techniques, the main discriminating factor is the number of micro-organisms. Theoretically, it would be possible to calculate the number of labelled molecules of the radiopharmaceutical bound to bacteria, relying on the molecular weight and specific activity of the radiopharmaceutical, the number, size and surface-to-volume ratio of bacteria. Another important aspect to consider for bacteria imaging is the host response, particularly on macrophage and monocyte reaction, which differ between living beings and tissues and psychological/metabolic condition of the host [9].

For this purpose, in chapter 4, the development of new radiopharmaceutical is described as new infection imaging probe. Ciprofloxacin dithiocarbamate (CiproCS<sub>2</sub>) has been chosen as candidate molecule and, after radiolabelling with  $^{99m}\text{Tc}$ , it was tested in vitro and in vivo for targeting *S. aureus* and *E. coli* induced infection in a tissue-cage mouse model and comparing it with three well-known radiopharmaceuticals,  $^{99m}\text{Tc}$ -UBI,  $^{99m}\text{Tc}$ -Ciprofloxacin and  $^{111}\text{In}$ -DTPA-biotin.

In the chapter 5, a similar approach is reported, but this time, the molecule of interest is the polymyxin B, an antimicrobial peptide that binds the lipopolysaccharide (LPS) of Gram-negative bacteria, with the aim to specifically image infections caused by Gram-negative.  $^{99m}\text{Tc}$ -polymyxin B was studied both in vitro, using several bacterial strains, and in vivo by inducing infection in the right thigh of mice.

Currently, radiolabelled WBCs are the nuclear medicine gold standard for the diagnosis of infections and inflammatory disorders, including osteomyelitis (OM) or prosthetic joint infections (PJI).

WBCs was firstly radiolabelled with  $^{111}\text{In}$ -oxine during 70s, but showed several drawbacks such as poor image quality or cell toxicity. Therefore, new methodologies were then tested to replace  $^{111}\text{In}$ -oxine until  $^{99m}\text{Tc}$ -HMPAO entered in clinical practice for ex-vivo WBCs labelling because of better image quality, less toxicity and isotope availability. Among tested agents, (S<sub>3</sub>CPh)<sub>2</sub>(S<sub>2</sub>CPh)-complex (SSS-complex) seemed

to be promising as a valid alternative to  $^{99m}\text{Tc}$ -HMPAO, but no systematic studies have ever been published to show the binding kinetics and specificity of this complex.

To this purpose, the chapter 6 deals with the radiolabelling of SSS-Complex with  $^{99m}\text{Tc}$ , evaluating its radiochemical purity, stability as well as the binding kinetics and specificity for blood cells, including granulocytes, lymphocytes and platelets, compared to  $^{99m}\text{Tc}$ -HMPAO.

Once radiolabelled WBCs, they specifically detect the granulocytes in early phases of infection, considering them as a surrogate marker of neutrophil-mediated infections. Indeed, the over time increased chemotaxis and vascular permeability as a consequence of infective processes, result in an exalted recruitment of granulocytes to the site of infection. The ability specifically to image granulocytes migration through the infected site depends highly from the modality of acquisition and the interpretative criteria adopted. The Infection and Inflammation committee of the European Association of Nuclear Medicine (EANM) developed criteria how to correctly label the leukocytes and provided suggestions regarding the acquisition protocols and interpretation criteria of this examination in musculoskeletal infections. Considering that the recruitment of granulocytes at the site of infection is a dynamic process, it is recommended to acquire images at different time points in order to reproduce the physiology [10]. By using the correct strategies, it is possible to discriminate an infection from an aseptic inflammation reaching an accuracy of around 90% that further increases when SPECT/CT is added to planar images for localization of the infectious process (in or outside the bone) [11].

However, the procedure to label WBCs is intensively time consuming and has high risk of cell contamination. Hence, a commercial and disposable sterile kit, called Leukokit<sup>®</sup>, has been developed to make easier the WBCs isolation from patient [12]. Firstly in this kit, poly(O-2-hydroxyethyl)starch (HAES-steril 10%, HES) has been routinely used as a sedimentation agent to remove erythrocytes (RBCs) from WBCs, but HES is now no commercially available and Gelofusine has been proposed as substitute sedimentation agent.

The chapter 7 is focused on the evaluation of Gelofusine availability in terms of red blood cells (RBCs) separation, recovered WBCs, RBCs and platelets contaminations, viability and chemotactic properties of neutrophils. After the introduction of Gelofusine in the Leukokit<sup>®</sup>, the second goal is the complete validation of “new” Leukokit<sup>®</sup> making a comparison with the “old” Leukokit<sup>®</sup> in terms of WBCs labelling efficiency, recovery yield, and diagnostic accuracy in patients with suspected infections.

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